

Application No.: Not Yet Assigned

Docket No.: 20793/0204562-US0

REMARKS

Claims 1-33 as presented in the underlying International Application No. PCT/EP2004/053659 have been canceled, and add new claims 34-66 have been added to adapt the claims of the underlying international application to U.S. practice. Appropriate amendments were made to the specification and abstract for the same purpose. It is respectfully submitted that no new matter has been added.

In view of the above amendment, issuance of a prompt and favorable Official Action is respectfully requested.

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Respectfully submitted,

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CROSS-REFERENCE TO PRIOR APPLICATION

FIELD OF THE INVENTION

[0002] Moreover, the invention relates to a method for the shading correction of at least one lens that is present in the microscope system and that defines an illumination field, comprising at least one light source that emits an illuminating light beam that illuminates a specimen through the lens and comprising at least one detector.

[0003] U.S. Pat. No. 6,355,919 discloses a method for calibrating a scanning microscope. Here, the calibration of the scanning microscope can be carried out as often as desired. The calibration means are arranged in a plane of an intermediate image and can be scanned by the scanning light beam. The calibration means are arranged outside

the actual image field and are configured as reference structures. However, this does not allow a compensation of the image field curvature.

SUMMARY

[0004] ~~The invention is based on the object of creating. It is an object of the present invention to provide~~ a microscope system with which an image and an illumination can be implemented in order to compensate for a correction of the edge shading caused by the image field curvature.

[0005] ~~The object is achieved by a microscope system comprising the features of Claim 1.~~

[0006] ~~The invention is based on the object of creating. It is another, alternative object of the present invention to provide~~ a method with which shading effects of the lens of a microscope system can be eliminated.

[0007] ~~The envisaged object is achieved by a method having the features of claim 17.~~

[0008] ~~It is advantageous for the microscope system to be provided with. In an embodiment the present invention provides~~ a microscope system having at least one lens that is present in the microscope system and that defines an illumination field. Furthermore, at least one light source is provided in the microscope system so as to emit an illuminating light beam that illuminates a specimen through the lens. By the same token, at least one detector is present that, pixel-by-pixel, detects a detection light beam coming from the specimen. An electronic circuit downstream from the detector serves to process the image data captured by the detector. A wavelength-dependent brightness distribution of the illumination field of the lenses present in the microscope system is saved in a memory unit. An actuatable element is provided in the illuminating light beam and it controls the intensity of the illuminating light beam pixel-by-pixel as a function of the stored, wavelength-dependent brightness distribution in such a way that the

illumination field is homogeneously illuminated. The electronic circuit computes the saved wavelength-dependent brightness distribution pixel-by-pixel in such a way that a homogeneously illuminated image field is formed.

[0009] The actuatable element in the illuminating light beam is an LCD matrix whose individual pixels are actuated according to the stored, wavelength-dependent brightness distribution. In one embodiment, the detector is a CCD chip.

[0010] In another embodiment, a scanning device is provided in the illuminating light beam of the microscope system and it conducts the illuminating light beam pixel-by-pixel over or through the specimen. The actuatable element in the illuminating light beam is an acousto-optic element that can be actuated as a function of the wavelength-dependent brightness distribution saved in the memory unit in such a way that the illumination field consisting of the individual pixels has a homogeneous brightness distribution. The acousto-optic element is an AOTF (Acousto-Optic Tunable Filter) or an AOBS (Acousto-Optic Beam Splitter) or an AOM (Acousto-Optic Modulator).

[0011] The microscope system can be equipped with different light sources such as, for example, a laser, a multiline laser or a laser that emits a continuous wavelength spectrum.

[0012] If a laser is used with the scanning device, the detector of the microscope system comprises at least one light-sensitive element that serially captures the pixels of the illumination field on the specimen. The electronic circuit combines the individual pixels to form an image field that can be computed with the appropriate wavelength-dependent brightness distribution.

[0013] ~~The~~In an embodiment the present invention provides a method for the shading correction is characterized by including the following steps:

- saving the wavelength-dependent brightness distribution in a RAM table;


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image field curvature. This phenomenon applies to individual objectives as well as to entire microscope systems and leads to a shading effect in the captured images. Figure 4a shows an image field 40. Each image field 40 becomes darker and darker as measured starting from a mid-point 41, that is to say, the intensity of the captured image field 40 or else the intensity of the illumination field decreases from the mid-point 41 towards the outside. This drop in intensity from the mid-point 41 towards the outside is likewise dependent on the wavelength. The image field 40 is, for example, rectangular and the intensity decreases towards a first and second long sides 42a and 42b respectively, and towards a first and second short sides 43a and 43b. An actuatable element 13 is provided in the illuminating light beam 5 and in the detection light beam 12. The actuatable element 13 is connected to an electronic circuit 14 that is provided with a memory unit 15. A wavelength-dependent brightness distribution such as, for example, in Figure 4a of the illumination field of the lenses 9 present in the microscope system is saved in the memory unit 15. By the same token, the electronic circuit 14 can also be connected to the lens 9 or to the revolving nosepiece, so that the electronic circuit 14 continuously receives information about the lens 9 that is momentarily located in the illuminating light beam or in the detection light beam. It is clear to any person skilled in the art that different lenses exhibit different shading. The electronic circuit 14 serves to control the intensity of the illuminating light beam 4 pixel-by-pixel as a function of the stored wavelength-dependent brightness distribution. The actuatable element 13 is controlled with the stored wavelength-dependent brightness distribution in such a way that the illumination field 46 (see Figure 4b) is homogeneous and does not display any drop in the intensity towards the long sides 42a, 42b and/or towards the short sides 43a and 43b. The detection light beam 12 coming from the specimen 10 is computed with the wavelength-dependent brightness distribution stored in the memory unit 15 in such a way that a homogeneously illuminated image field 40 is created. The detection light beam 12 coming from the specimen is detected pixel-by-pixel by the detector 20, which is a CCD chip. This data is fed to the electronic circuit 14 which then compares the stored wavelength-dependent brightness distribution to the measured wavelength-dependent brightness distribution as a function of the wavelength.

circuit 14. At least one peripheral device 27 is associated with the electronic circuit 14. This peripheral device 27 can be, for example, a display on which the user receives information about the setting of the scanning microscope or about the current setup and can also obtain the image data in graphic form. Moreover, the memory unit 15 is connected to the electronic circuit 14 and, as already described, this is where the wavelength-dependent brightness distribution – shown, for example, in Figure 3b – of the illumination field 46 of the lenses 9 present in the microscope system 1 is saved.

[0018] The SP module 23 spatially, spectrally splits the detection light beam 12 with a prism 31. Another possibility for spectral splitting is the use of a reflecting diffraction grating or a transmission diffraction grating. The spectrally split light fan 32 is focused with the focusing lens 33 and subsequently strikes a mirror diaphragm arrangement 34, 35. The mirror diaphragm arrangement 34, 35, the means for spectral, spatial splitting, the focusing lens 33 and the detectors 36 and 37 are referred to together as the SP module 23 (or multi-band detector).

[0019] The scanning device 16 conducts the illuminating light beam 5 pixel-by-pixel over or through the specimen 10. The actuatable element 13 in the illuminating light beam 5 is an acousto-optic element that can be actuated as a function of the wavelength-dependent brightness distribution saved in the memory unit 15 in such a way that the illumination field 46 consisting of the individual pixels has a homogenous brightness distribution. The acousto-optic element 13 is an AOTF (Acousto-Optic Tunable Filter) or an AOBS (Acousto-Optic Beam Splitter) or an AOM (Acousto-Optic Modulator). The light source 3 consists of at least one laser that generates the illuminating light beam 5. The at least one laser can be a multiline laser. By the same token, it is conceivable for the laser to emit a continuous wavelength spectrum so that the specimen 10 is either illuminated with a continuous wavelength spectrum or the user selects any desired wavelengths from the continuous wavelength spectrum in order to illuminate the specimen 10.

[0020] Figure 3 shows a schematic depiction of another embodiment of the scanning microscope 100 with a control circuit 60 for changing the intensity of the illuminating light beam 5 coming from the light source 3. The same elements as already described for Figure 2 are designated with the same reference numerals. The light source 3, which is configured as a multiline laser, is provided with the control circuit 60 so that the intensity of the illumination light being emitted by the laser is controlled as a function of the stored wavelength-dependent brightness distribution. The illumination field 46 (see Figure 4b) is then illuminated homogeneously and does not exhibit any drop in the intensity towards the long sides 42a, 42b and/or towards the short sides 43a, 43b. The dyes present in the specimen 10 are excited by the illuminating light beam 5 and emit light in a characteristic spectral range that is specific to them. This light coming from the specimen defines a detection light beam 12. It passes through the lens 9, the scanning lens 19 and, via the scanning device 16, reaches a wavelength-selective element 63, traverses the latter without being influenced and, for example, via a detection pinhole 21, reaches at least one detector 20 that is configured as a photomultiplier. Other embodiments of the detector were already mentioned in the description pertaining to Figure 2.

[0021] Figure 5 shows a schematic depiction of the detector 20 which, in this embodiment, is configured as an area sensor 44 in order to detect the detection light beam 12 coming from the specimen 10. The area sensor 44 comprises multiple pixels $45_{1,1}$, $45_{1,2}$, $45_{1,3}$, ..., $45_{n,m-1}$, $45_{n,m}$, which are arranged on a surface. The light coming from the specimen 10 is imaged by the lens 9 onto the area sensor 44. The individual pixels $45_{1,1}$, $45_{1,2}$, $45_{1,3}$, ..., $45_{n,m-1}$, $45_{n,m}$ register the intensity, which is superimposed on the shading effect of the lens 19. The wavelength-dependent brightness distribution is saved in the memory unit 15 and the electronic circuit 14 compares the data captured by the detector 20 to the wavelength-dependent brightness distribution, so that the captured image field 40 is homogeneously illuminated at the wavelength λ_n .

[0022] Figure 6 shows a schematic depiction of the actuatable element 13 where the individual pixels $50_{1,1}$, $50_{1,2}$, $50_{1,3}$, ..., $50_{n,m-1}$, $50_{n,m}$ are actuated according to the brightness distribution for a given wavelength. Generally speaking, the gray values


associated with the pixels $50_{1,1}$, $50_{1,2}$, $50_{1,3}$, ..., $50_{n,m-1}$, $50_{n,m}$ constitute the shading effect caused by the lens 9. This is the negative depiction for the actuation of the individual pixels $50_{1,1}$, $50_{1,2}$, $50_{1,3}$, ..., $50_{n,m-1}$, $50_{n,m}$. This means that the pixels $50_{1,1}$, $50_{1,2}$, $50_{1,3}$, ..., $50_{n,m-1}$, $50_{n,m}$ are actuated inversely as a function of the gray value, that is to say, the darker the pixels $50_{1,1}$, $50_{1,2}$, $50_{1,3}$, ..., $50_{n,m-1}$, $50_{n,m}$ are, the less the shading caused by the actuation is. As already mentioned, the actuation of the actuatable element 13 is such that the illumination field 46 on the specimen 10 is homogeneously illuminated.

[0023] A laser beam illuminates the specimen 10 in a meander-like manner, so that the specimen 10 is illuminated, that is to say, the illumination field is covered consecutively with a plurality of image points. A scanning device 16 conducts the illuminating light beam 5 pixel-by-pixel over or through the specimen 10 in order to yield a homogeneously illuminated illumination field 46; the actuatable element 13 in the illuminating light beam 5 is an acousto-optic element 13. As shown in the depiction of Figure 5, the acousto-optic element 13 is likewise actuated inversely. This means that the greater the gray value due to the shading is, the higher the intensity of the laser beam has to be when it reaches this position. This takes place as a function of the wavelength-dependent brightness distribution saved in the memory unit 15 in such a way that the illumination field 46 consisting of the individual pixels has a homogenous brightness distribution.

[0024] As already mentioned, the shading effect occurs. The shading effect can be described by $f(x, y, \lambda)$. Here, x is the X-position and y is the Y-position of the individual pixel in the illumination field. It is especially advantageous for the wavelength-dependent brightness distribution to be depicted as a model. The wavelength-dependent brightness distribution or the attenuation for each wavelength can be expressed as a functional. Here,

$$F(x, y) = a + bx + cy + dxy + ex^2 + fy^2$$

is seen as a good approximation, whereby the coefficients depend on the wavelength. The following applies: $a(\lambda)$, $b(\lambda)$, $c(\lambda)$, $d(\lambda)$, $e(\lambda)$, $f(\lambda)$. It is sufficiently familiar to a person

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skilled in the art that higher polynomial models can also be used. An illumination pattern that corrects the shading effect is then:

$$G(x, y) = F^{-1} (x - x_0, y - y_0)$$

which, in this context, raises the illumination characteristics where the lens causes a damping, while the shift x_0, y_0 stands for the quality of the adjustment. If the laser does not run towards at the specimen 10 centrally in the illumination field 46, then this misalignment can also be taken into account in the determination of the wavelength-dependent brightness distribution. The system then uses the appropriate correction for the illumination of the specimen 10.